PRELIMINARY AND SHORT REPORT

A SIMPLE AID IN THE MICROSCOPIC DETECTION OF MELANIN

MILLARD A. TROXELL, M.D.*

Cedar Rapids, Iowa

METHOD

1. A specimen of cutaneous tissue is obtained in the usual manner, fixed in ten per cent formalin, and embedded in paraffin. It can be studied at any later time.

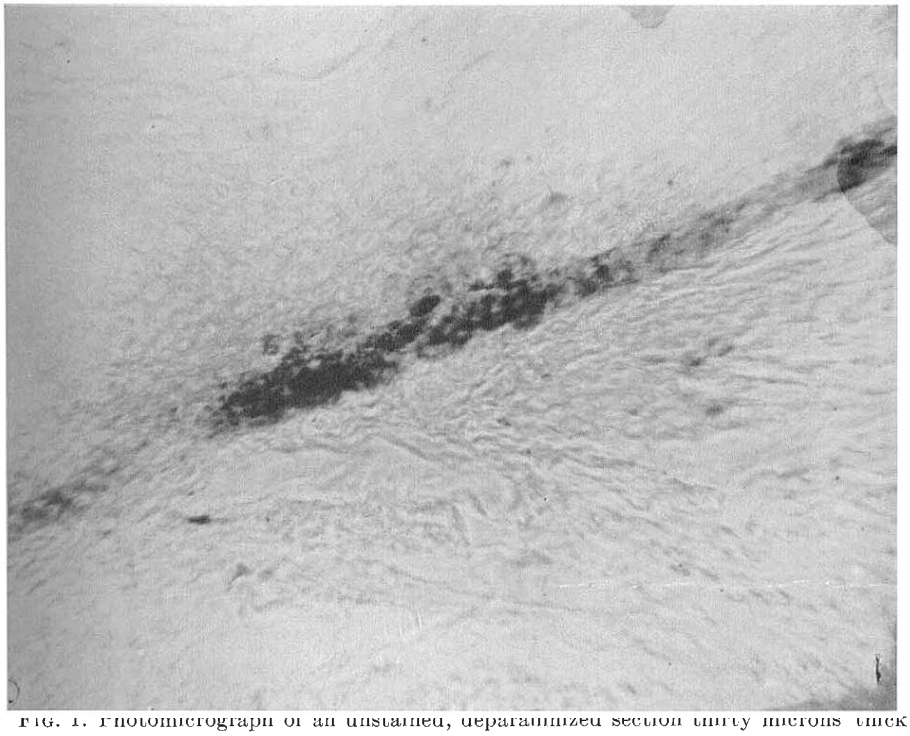

FIG. 1. Photomicrograph of an unstained, deparaffinized section thirty microns thick; ×430; 560 millimicron light filter.

Many granules and clumps of pigment may be seen in the basal and suprabasal layers. The epidermal pigment seems otherwise sparse and finely granulated. The dermal pigment also seems sparse, but occurs in granules and clumps in isolated small areas, presumably within chromatophores.

2. An unstained, deparaffinized section cut thirty microns thick is mounted with clarite and examined under the geologic polariscope. Plastic polarizing discs may be used with

* Formerly, medical fellow, the University of Minneosta, Division of Dermatology, H. E. Michelson, M.D., Director, and, Ancker Hospital, St. Paul, Minnesota, Department of Dermatology, J. F. Madden, M.D., Director.

Received for publication August 24, 1953.

FIG. 2. Photomicrograph same as Figure 1 except for the addition of a neutral density filter.

The potentialities of proper light filtration is illustrated. The pigment granules are more distinct, appear to be in greater number, are evident higher in the epidermis, and many more chromatophores are seen.

the medical microscope (1) as a compromise. Crystalline matter will generally show birefringence (2), while melanin will not.

3. The same section is examined under the brightfield microscope, which Richards (3) considers to be the best microscope for the examination of colored specimens. The tungsten light produced by the microscope lamp contains a greater proportion of the longer wave lengths than sunlight but may be changed to daylight quality by filtering through blue glass (3). This is routinely done in medical microscopy. However, *limiting the light with a suitable filter to the yellow-green will give a better image with achromatic objectives, because that is the region for which the objectives are corrected and also the region of greatest sensitivity of the eye* (3).

In examining unstained sections of skin under the ordinary brightfield microscope with artificial light filtered through blue glass, the results are most disappointing and it is difficult even to locate the sections. The substitution of a yellow-green filter and/or addition of neutral density filters sharply increases the definition of the structures in the section,

and melanin shows up especially well if present. If greater definition is desired, a yellow-green filter which transmits a slightly different wave length can be substituted. A few trial combinations of yellow-green and/or neutral density filters can produce very satisfying results, whether or not photomicrography is intended.

4. These light filters also can be used in the examination of routine, stained sections. The appropriate filters mask off the dark colored stains which obscure melanin. Perhaps the opposing wave length on the classical color spectrum wheel is obliterated.

5. This method does not absolutely identify melanin, and should be used as an adjunct to recognized silver stains for the identification of pre-formed melanin. In distinction, the "dopa" stain is used to demonstrate the capacity for the formation of melanin.

6. The two accompanying photomicrographs differ only in the filtration of the incident light. Tissue was obtained from a pigmented spot in the lip of a patient who also had generalized intestinal polyposis (Syndrome of Peutz).

COMMENT

One need not be searching for melanin, nor engaged in research, to enjoy the use of light which is properly filtered for the specimen which is to be examined. The microscopist is urged to purchase some neutral density filters, and at least a few filters in the 5000 to 6000 Ångstrom range. These filters are available (Corning Glass Works, Corning, New York; Eastman Kodak Company, Rochester 4, New York) in glass and gelatin to meet most needs (3).

CONCLUSIONS

1. A simple technic to aid in the microscopic detection of melanin is described.
2. Its potentialities in the detection of other substances is suggested.

REFERENCES

1. LEVER, W. F.: Histopathology of the Skin, Philadelphia, J. Lippincott Co., 1949, p. 25.
2. LICHTMAN, A. L., AND MCDONALD, J. R.: Birefringence in tissues, Arch. Path. **42:** 69-80 1946.
3. RICHARDS, O. W.: When to use special microscopes, in Uber, F. M.: Biophysical Research Methods, New York, Interscience Pub., Inc., 1950, p. 343-380.